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Morphological changes in spiral ganglion cells after intracochlear application of brain-derived neurotrophic factor in deafened guinea pigs

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ABSTRACT

When guinea pigs are deafened with ototoxic drugs spiral ganglion cells (SGCs) degenerate progressively. Application of neurotrophins can prevent this process. Morphological changes of rescued SGCs have not been quantitatively determined yet. It might be that SGCs treated with neurotrophins are more vulnerable than SGCs in cochleae of normal-hearing guinea pigs. Therefore, the mitochondria and myelinisation of type-I SGCs were studied and the perikaryal area, cell circularity and electron density were determined. Guinea pigs were deafened with a subcutaneous injection of kanamycin followed by intravenous infusion of furosemide. Brain-derived neurotrophic factor (BDNF) delivery was started two weeks after the deafening procedure and continued for four weeks. Four cohorts of cochleae were studied: (1) cochleae of normal-hearing guinea pigs; (2) of guinea pigs two weeks after deafening; (3) six weeks after deafening; (4) cochleae treated with BDNF after deafening. The deafening procedure resulted in a progressive loss of SGCs. Six weeks after deafening the size of mitochondria, perikaryal area and cell circularity of the remaining untreated SGCs were decreased and the number of layers of the myelin sheath was reduced. In the basal part of the cochlea BDNF treatment rescued SGCs from degeneration. SGCs treated with BDNF were larger than SGCs in normal-hearing guinea pigs, whereas circularity had normal values and electron density was unchanged. The number of layers in the myelin sheath of BDNF-treated SGCs was reduced as compared to the number of layers in the myelin sheath of SGCs in normal-hearing guinea pigs. The morphological changes of SGCs might be related to the rapid loss of SGCs that has been reported to occur after cessation of BDNF treatment.

1. Introduction

One of the factors explaining the variability in perceptual performance of cochlear implant users might be the amount of surviving spiral ganglion cells (SGCs) in relation to the extent and duration of the sensorineural hearing loss. Animal studies have shown that loss of inner hair cells (IHCs) results in progressive loss of SGCs (Ylikoski et al., 1974; Webster and Webster, 1981), possibly as a consequence of loss of neurotrophic support (Lefebvre et al., 1992). Most studies report a decrease in perikaryal area of SGCs after hair cell loss (Staecker et al., 1996; Leake et al., 1999; Shepherd et al., 2005; Richardson et al., 2005). Behavioral studies in monkeys have presented strong evidence that a greater survival of SGCs is associated with improved performance with a cochlear implant (Pfingst et al., 1981; Pfingst and Sutton, 1983). Studies in rats and cats have shown that progressive loss of SGCs reduces the efficacy of electrical stimulation, as measured by electrically evoked auditory brainstem responses (Hall, 1990; Miller et al., 1994; Hardie and Shepherd, 1999; Shepherd et al., 2004). Although there is no explicit clinical evidence from post-mortem studies that enhanced SGC survival results in a better performance with a cochlear implant (Nadol et al., 2001; Khan et al., 2005), it might be clinically important to preserve the integrity of the peripheral neural system in the cochlea of cochlear implant candidates and recipients.

A potential way to preserve SGCs after deafening via ototoxic drugs, is the exogenous intracochlear application of neurotrophic factors (for overviews, see Miller et al., 2002; Gillespie and Shepherd, 2005; Pettingill et al., 2007). Intracochlear application of neurotrophic factors, such as neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF), prevents loss of auditory

Abbreviations: ABR, auditory brainstem response; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CNTF, ciliary-derived neurotrophic factor; dB, decibel; ID, inner diameter; IHC, inner hair cell; IM, intramuscular; IV, intravenous; NGF, nerve growth factor; NT-3, neurotrophin 3; OHC, outer hair cell; PBS, phosphate-buffered saline; SGC, spiral ganglion cell; 2wdu, two-weeks deaf untreated; 6wdu, six-weeks deaf untreated; 6wdBDNF, six-weeks deaf BDNF treated

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neurons in the guinea pig (Staecker et al., 1996; Miller et al., 1997). When neurotrophic treatment was started after degeneration had set in, 2–6 weeks after deafening, the remaining SGCs could still be rescued (Gillespie et al., 2004; Yamagata et al., 2004; Richardson et al., 2005; Wise et al., 2005; Miller et al., 2007). According to the neurotrophin hypothesis and based upon findings in other sensory systems (Montero and Hefti, 1988; Mansour-Robaey et al., 1994), it is expected that cessation of BDNF treatment results in degeneration of the SGCs. Gillespie et al. (2003) have confirmed this expectation. Remarkably, they reported an accelerated degeneration of SGCs after cessation of BDNF treatment as compared to degeneration without BDNF. This abnormally rapid degeneration suggests that the SGCs have changed during BDNF treatment and that rescued SGCs are more vulnerable than SGCs at the start of treatment. Some studies qualitatively describe BDNF-treated SGCs with terms as: "large", "round" and "healthy" in order to assess the size, shape, and condition of the SGCs (Shah et al., 1995; Gillespie et al., 2004; Miller et al., 2007). Although two studies have quantitatively shown that BDNF-treated SGCs in deafened guinea pigs are larger than untreated SGCs (Shepherd et al., 2005; Richardson et al., 2005) and one study reported a reduction in the number of individual layers in the myelin sheath of SGCs treated with BDNF and acidic fibroblast growth factor (Glueckert et al., 2008), the shape and condition of the SGCs rescued with BDNF have not been quantitatively determined.

The aim of this study was to characterize and quantify morphological changes in SGCs after deafening and subsequent BDNF treatment. Light microscopy was used to examine the amount of degeneration of SGCs after delayed BDNF treatment. Electron microscopy was used to describe the morphology of the SGCs in detail and quantify their size, circularity and electron density.

2. Materials and methods

2.1. Animals and experimental design

Albino female guinea pigs (strain: Dunkin Hartley; weighing 250–350 g) were purchased from Harlan Laboratories (Horst, The Netherlands) and housed in the animal care facility of the Rudolf Magnus Institute of Neuroscience. All animals had free access to both food and water and were kept under standard laboratory conditions. Lights were on between 7:00 am and 7:00 pm. Temperature and humidity were kept constant at 21 °C and 60%, respectively.

We used one control group and two experimental groups of animals. In these groups both left and right cochleae were analyzed using light and electron-microscopical procedures. The control group consisted of four normal-hearing guinea pigs (*normal*). The right cochleae of these animals were implanted with a cannula two weeks after a sham deafening procedure. Phosphate-buffered saline (PBS) was infused into the cochleae for four weeks, and the animals were sacrificed for histology immediately after finishing the PBS infusion. The first experimental group contained three guinea pigs sacrificed for histology two weeks after deafening (two-weeks deaf untreated, *2wdu*), of which both the left and right cochlea were used. The second experimental group consisted of six animals, sacrificed six weeks after deafening, of which the left cochleae were untreated (six-weeks deaf untreated, *6wdu*). The right cochleae of these animals were implanted with a cannula two weeks after deafening and treated with BDNF for four weeks (six-weeks deaf BDNF-treated, *6wdBDNF*). These animals were sacrificed for histology immediately after finishing the BDNF treatment. Summarizing, four cohorts of cochleae were studied (*normal*, *2wdu*, *6wdu*, and *6wdBDNF*). All implanted animals included in this study had an open cannula at termination and were without any trace of otitis media. All experimental procedures were approved by the University's Committee on Animal Research (DEC-UMC # 03.04.036).

2.2. Deafening procedure and ABRs

Animals were anesthetized with xylazine (Sedamun®, i.m. 10 mg/kg) and ketamine (Ketanest®, i.m. 40 mg/kg). Before the deafening procedure, three stainless steel screws (8.0 × 1.2 mm) were inserted into the skull bone to record auditory brainstem responses (ABRs). The screws were inserted 1 cm posterior to bregma, 2 cm anterior to bregma and 1 cm lateral from bregma (Mitchell et al., 1997). Before, during and after deafening we recorded ABRs to monitor hearing thresholds. Measurements were performed in a sound-attenuated chamber. Broadband click stimuli consisting of biphasic rectangular pulses (100 μs/phase) were presented in free field using a tweeter (Fane J-104) positioned 10 cm above the unanesthetized guinea pig. Stimulus generation and signal acquisition were controlled with custom-written software and a personal computer (for details, see Versnel et al., 2007). Stimuli were presented from 86 dB above threshold in normal-hearing animals down to threshold in 10 dB steps. Threshold was defined as the sound level at which the ABR was just noticeable upon visual inspection of the response.

When initially normal hearing thresholds were confirmed, kanamycin (400 mg/kg) was injected subcutaneously followed by slow intravenous infusion of furosemide (100 mg/kg) as a loop diuretic. This procedure, first described by West et al. (1973) using ethacrynic acid (40 mg/kg) as the loop diuretic, has been shown to eliminate almost all cochlear hair cells. Gillespie et al. (2003) and Versnel et al. (2007) confirmed this procedure using furosemide instead of ethacrynic acid. For the intravenous infusion of furosemide, the left jugular vein was exposed and a catheter was inserted. A successful insertion was confirmed with withdrawal of blood into the catheter. The control animals received isotonic saline (subcutaneously and intravenously), instead of kanamycin and furosemide. After deafening, the ABRs were measured on days 1, 7 and 14 to assess the extent of hearing loss. All animals of the experimental groups included in this study demonstrated a threshold shift of $\geqslant$50 dB, measured 14 days after deafening.

2.3. Cannula implantation and BDNF treatment

Two weeks after the deafening procedure, six deafened animals and the four control animals were implanted with a cannula in the right cochlea. The cannula consisted of medical vinyl tubing (90 mm, ID: 0.8 mm) with a tip of polyimide tubing (11 mm, ID: 0.12 mm); the total volume was ~40 μl. Alzet mini-osmotic pumps (model 2004; flow rate 0.25 μl/h; reservoir 200 μl) were filled with BDNF (PeproTech®) solution (100 μg/ml), and then incubated in sterile saline for 48 h at 37 °C to guarantee a constant flow rate at implantation. The BDNF concentration of 100 μg/ml was chosen because it is in the range of dosages (50–100 μg/ml) that have been proven to be effective in several studies; Gillespie et al. (2003, 2004) infused 62.5 μg/ml, Shepherd et al. (2005) 62.5 μg/ml, Wise et al. (2005) 50 μg/ml, and Miller et al. (2007) used 100 μg/ml. Cochleae were treated with BDNF for four weeks. For the normal-hearing animals, the mini-osmotic pumps were loaded with PBS, pH 7.4. Bovine serum albumin (BSA, 1%) was added to the BDNF and PBS solutions.

The animals were anesthetized with xylazine and ketamine, and the right bulla was exposed retro-auricularly. A small hole was drilled to visualize the cochlea. The round window membrane was perforated and the tip of the cannula was inserted into the scala tympani through the round window (Prieskorn and Miller,